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Acute Toxicities of Cd^{2+}, Cr^{+6}, Hg^{2+}, Ni^{2+}. and Zn^{2+} to Estuarine Macrofauna

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16. ABSTRACT

Abstract. Static acute toxicity bioassays were conducted at 20°C and 20 o/oo salinity with $CdCl_2 \cdot 2\frac{1}{2}H_2O$, K_2CrO_4, $HgCl_2$, $NiCl_2 \cdot 6H_2O$, and $ZnCl_2$ using adults of starfish, *Asterias forbesi;* sandworm, *Nereis virens;* hermit crab, *Pagurus longicarpus;* softshell clam, *Mya arenaria;* mudsnail, *Nassarius obsoletus;* and mummichog, *Fundulus heteroclitus,* a fish. Concentrations (mg/L metal) fatal to 50% of the organisms in 168 hr ranged from 0.004 (clam) to 0.8 (mummichog) for mercury; 0.7 (clam, worm, crab and starfish) to 40.0 (mummichog) for cadmium; 0.2 (crab) to 52.0 (mummichog) for zinc; 0.7 (sandworm) to 44.0 (mummichog) for hexavalent chromium; and 13.0 (starfish) to 150.0 (mummichog) for nickel. Biocidal action was restricted to a relatively narrow range for all species-metals combinations tested: *i.e.*, mean LC-75/LC-25 ratios for individual metals at 168 hr ranged between 2.82 (Zn) and 6.02 (Cd); for individual species this ratio extended from 2.76 (fish) to 4.46 (clam). It appears that acute toxicity evaluation of potentially hazardous metals in saline environments requires utilization of at least several representative species from divergent taxonomic and ecological niches.

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Acute Toxicities of Cd^{2+}, Cr^{+6}, Hg^{2+}, Ni^{2+} and Zn^{2+} to Estuarine Macrofauna

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Abstract. Static acute toxicity bioassays were conducted at 20°C and 20 o/oo salinity with $CdCl_2 \cdot 2\frac{1}{2}H_2O$, K_2CrO_4, $HgCl_2$, $NiCl_2 \cdot 6H_2O$, and $ZnCl_2$ using adults of starfish, *Asterias forbesi;* sandworm, *Nereis virens;* hermit crab, *Pagurus longicarpus;* softshell clam, *Mya arenaria;* mudsnail, *Nassarius obsoletus;* and mummichog, *Fundulus heteroclitus,* a fish. Concentrations (mg/L metal) fatal to 50% of the organisms in 168 hr ranged from 0.004 (clam) to 0.8 (mummichog) for mercury; 0.7 (clam, worm, crab and starfish) to 40.0 (mummichog) for cadmium; 0.2 (crab) to 52.0 (mummichog) for zinc; 0.7 (sandworm) to 44.0 (mummichog) for hexavalent chromium; and 13.0 (starfish) to 150.0 (mummichog) for nickel. Biocidal action was restricted to a relatively narrow range for all species-metals combinations tested: *i.e.*, mean LC-75/LC-25 ratios for individual metals at 168 hr ranged between 2.82 (Zn) and 6.02 (Cd); for individual species this ratio extended from 2.76 (fish) to 4.46 (clam). It appears that acute toxicity evaluation of potentially hazardous metals in saline environments requires utilization of at least several representative species from divergent taxonomic and ecological niches.

Salts of various heavy metals and other potentially hazardous materials are being discharged in ever-increasing amounts into saline environments from mining operations, metals processing facilities, chemical industries, and numerous other sources (McKee and Wolf 1963, Bernhard and Zattera 1975, Goldberg 1976). In one near-classic case, mercury-containing wastes discharged into Minamata Bay, Japan, were taken up by marine fish and shellfish and subsequently implicated as the causative agent of more than 100 human deaths and congenital birth defects (Irukayama 1967). In general, however, effects of heavy metals upon marine and estuarine organisms are poorly-documented when compared to those for freshwater biota (Eisler 1973, Eisler and Wapner 1975).

This account reports on comparative acute lethalities of salts of cadmium, hexavalent chromium, mercury, nickel, and zinc to adults of six common species of estuarine macrofauna under controlled conditions; specifically, we observed survival during a period of 168 hr of asteroid echinoderms, gastropod molluscs, bivalve molluscs, decapod crustaceans, polychaete annelids, and cyprinodontiform teleosts. It is part of a continuing series of investigations conducted by this laboratory on toxicological hazards of selected waste components to representative species of marine biota.

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Methods

Organisms used were adults of six species of estuarine macrofauna: starfish, *Asterias forbesi;* sandworm, *Nereis virens;* hermit crab, *Pagurus longicarpus;* softshell clam, *Mya arenaria;* mud-snail, *Nassarius obsoletus;* and mummichog, *Fundulus heteroclitus*, a teleost. All test species, except sandworm, were collected during late spring from lower Narragansett Bay, R.I. Sandworms were purchased from a local bait dealer who received twice weekly shipments of *Nereis* from a supplier in Maine. Prior to testing, individual species were confined to fiberglass tanks containing 225 L of raw sea water of 20 0/00 salinity (adjusted downwards from 30 0/00 with distilled water), pH 7.8 ± 0.2, dissolved oxygen >4.0 mg/L, and a temperature of 20.0 ± 0.5°C. Sea water in holding aquaria was from a reservoir of 1000 L capacity recirculated through glass wool-gravel filters; about 500 L of reservoir water was replaced weekly. Highest concentrations of selected metals in filtered sea water from this system, as determined periodically using a Perkin-Elmer Model 303 atomic absorption spectrophotometer (AAS) and methods in the accompanying manual, were always less than 0.001 mg/L for Cd, 0.004 mg/L for Zn, 0.006 mg/L for Cr, and 0.010 mg/L for Ni. Background levels of Hg were not measured. During holding, all organisms except clam fed actively on a prepared diet (LaRoche *et al.* 1970) offered daily. Clams were not fed at any time, but it is possible that food was available from the raw sea water medium. Under these conditions we observed that all test species survived for at least four months, with apparently normal growth and behavior.

For all species, mortality during holding was less than 5% during the first 48 hr and less than 1% afterwards. Feeding was discontinued on the 12th day. Results of preliminary toxicity studies that were conducted using teleosts, crustaceans, molluscs, and selected heavy metals salts indicated that starvation for 10 days *vs.* 48 hr did not markedly affect the results of 7-day toxicity studies. At day 14 each species was apportioned among widemouth glass jars containing 2 L of new raw sea water of identical pH, temperature, salinity, dissolved oxygen, and metals content experienced during acclimatization. Information on length, weight, and biomass of test organisms is shown in Table 1. A total of 30 toxicity tests were conducted, 6 species *vs.* 5 metals. For each test, every species was exposed to a minimum of 6 concentrations excluding controls, a minimum of 8 animals per concentration, 2 to 10 replicates per concentration, and 1 to 5 organisms per replicate. At least 60 animals of each species was utilized in every test, although most employed 80 or more (Table 1).

Compounds tested were reagent grades of $CdCl_2 \cdot 2\frac{1}{2}H_2O$, K_2CrO_4, $HgCl_2$, $NiCl_2 \cdot 6H_2O$, and $ZnCl_2$ dissolved in distilled water. The mercury stock solution contained 10 mg of Hg^{2+}/ml; all others were 50 mg of metal/ml. A concentration gradient series for each metal was prepared in distilled water so

Table 1. Length, weight, biomass, and numbers of organisms used per test in study on acute toxicities of 5 metals to 6 species of estuarine macrofauna

	Length (mm)		Wet weight (g)		Grams biomass per liter	Number per test	
	Mean	Range	Mean	Range		Min.	Max.
Snail	20.5[a]	19– 24	0.40[b]	0.3– 0.5	1.00	60	90
Crab	19.3[c]	15– 24	0.50	0.2– 1.0	1.25	60	80
Fish	49.3	36– 52	1.30	0.9– 2.0	1.30	60	84
Clam	42.0	38– 45	4.60[b]	2.0– 6.0	2.30	70	90
Sandworm	259.5	186–280	7.60	4.0–10.0	3.80	70	90
Starfish	34.3[d]	26– 42	11.20	5.0–18.0	5.60	64	80

[a] Apex to tip of siphonal canal
[b] Meats only
[c] Length of gastropod shell housing
[d] Madreporite to end of longest arm

Table 2. Loss of selected metals from synthetic seawater[a] solution of pH 7.8, 20 o/oo salinity, and 20°C during 96 hr

	Metal				
	Cd^{2+}	Cr^{6+}	Ni^{2+}	Zn^{2+}	Hg^{2+}
Initial concentration, mg/L	27.0	110.0	150.0	16.0	0.23
Final concentration in filtrate, mg/L[b]	27.0	110.0	139.0	14.2	0.17
Percent loss in 96 hr	0.0	0.0	7.3	11.2	26.1

[a] Zaroogian *et al.* (1969) formulation
[b] After passage through Whatman 42 paper

that addition of 10 to 20 ml of the diluent would produce the desired level in test aquaria; test concentrations were always within 5% of calculated values as determined by AAS. Final concentrations selected were based on results of preliminary acute toxicity bioassays with the 5 metals salts and limited numbers of test organisms. Metals were added to bioassay containers approximately 60 min after test organisms were introduced. Jars were not supplied with aeration.

Dead animals were removed and recorded every 24 hr for 168 hr. Concentrations of each metal lethal to 25% (LC-25), 50% (LC-50), and 75% (LC-75) of individual species were determined by graphical interpolation at different exposure periods (American Public Health Association 1971). In addition, for each species and metal tested, the highest concentration at which no deaths were observed (LC-0) as well as the lowest concentration that was fatal to all (LC-100) was recorded every 24 hr. Dissolved oxygen and pH measurements were made daily.

It is underscored that LC values were based on initial concentrations of metals added to the medium, and as such were probably conservative. They do not reflect known loss rates of individual metals from sea water with time as a result of physical, chemical, and biological factors. For example, we found that loss of Cd^{2+}, Cr^{+6}, Ni^{2+}, Zn^{2+}, and Hg^{2+} during 96 hr due to physicochemical processes, *i.e.*, loss from synthetic sea water media in 25 ml flasks without organisms as measured by AAS, ranged from zero for Cd^{2+} and Cr^{+6} to 26.1% for Hg^{2+} (Table 2). Most of the observed loss of metals occurred during the first 48 hr. Synthetic sea water is not strictly comparable in chemical properties to the raw sea water used herein. Nevertheless, results of preliminary toxicity tests with fishes, invertebrates, and selected compounds including metals, conducted in both synthetic sea water and raw sea water of identical thermosaline regimen indicated less than 10% difference in calculated LC-50 (96 hr) values.

Results

During the 168-hr test interval dissolved oxygen values were always >4.0 mg/L. Depressed pH values were recorded at some of the higher concentrations tested of Zn^{2+}, Ni^{2+}, and Cr^{+6}, but these were never greater than 0.6 unit. All organisms comprising the 30 control groups (n = 10/group) survived and appeared outwardly normal at 168 hr.

Interpolated LC-50 values at 24, 96, and 168 hr for each of the 5 metals and 6 test species are shown in Table 3; also shown for these intervals were observed LC-0 and LC-100 concentrations. In almost every instance there was a marked and progressive decline in LC values between 24 and 168 hr. LC-50 (168 hr)

Table 3. Concentrations of metals fatal to 0, 50, and 100% of test organisms at 3 time intervals. Tests conducted at 20°C and 20 o/oo salinity with clam (*Mya arenaria*), starfish (*Asterias forbesi*), crab (*Pagurus longicarpus*), sandworm (*Nereis virens*), snail (*Nassarius obsoletus*), and fish (*Fundulus heteroclitus*). Values are in mg metal added initially per liter seawater (ppm)

		Time interval (hr)								
		24			96			168		
Metal	Organism	LC-0	LC-50	LC-100	LC-0	LC-50	LC-100	LC-0	LC-50	LC-100
Hg	Clam	1.000	5.200	10.000	0.030	0.400	2.000	0.001	0.004	0.030
	Starfish	0.125	1.800	5.000	0.010	0.060	0.250	0.010	0.020	0.125
	Crab	1.000	2.200	5.000	0.010	0.050	0.125	0.010	0.050	0.125
	Sandworm	1.000	3.100	10.000	0.025	0.070	0.250	0.025	0.060	0.125
	Snail	20.000	32.000	50.000	0.500	32.000	50.000	0.100	0.700	5.000
	Fish	10.000	23.000	50.000	0.100	0.800	2.000	0.100	0.800	1.000
Cd	Crab	3.0	15.0	30.0	0.5	1.3	3.0	0.1	0.7	2.0
	Clam	5.0	32.0	50.0	1.0	2.5	25.0	0.1	0.7	5.0
	Starfish	50.0	71.0	100.0	5.0	7.1	10.0	0.1	0.7	5.0
	Sandworm	30.0	56.0	100.0	0.1	9.3	20.0	0.1	0.7	5.0
	Snail	100.0	175.0	300.0	25.0	35.0	50.0	10.0	16.0	25.0
	Fish	25.0	220.0	500.0	5.0	22.0	50.0	5.0	22.0	50.0
Zn	Crab	5.0	12.0	30.0	0.1	0.4	5.0	0.1	0.2	0.5
	Starfish	300.0	390.0	500.0	10.0	39.0	300.0	1.0	2.3	5.0
	Sandworm	4.5	20.0	30.0	4.5	8.1	15.0	1.5	2.6	4.5
	Clam	200.0	320.0	500.0	5.0	7.7	25.0	1.0	3.1	5.0
	Snail	50.0	150.0	300.0	10.0	50.0	300.0	5.0	7.4	30.0
	Fish	15.0	125.0	180.0	15.0	60.0	120.0	10.0	52.0	120.0

Cr	Sandworm	35.0	80.0	200.0	0.5	2.0	5.0	0.1	0.7	5.0
	Crab	20.0	31.0	50.0	5.0	10.0	20.0	1.0	2.7	20.0
	Clam	100.0	225.0	500.0	25.0	57.0	100.0	5.0	8.0	50.0
	Starfish	300.0	540.0	1000.0	20.0	32.0	50.0	5.0	10.0	20.0
	Snail	150.0	390.0	1000.0	75.0	105.0	150.0	5.0	10.0	20.0
	Fish	200.0	>200.0	>200.0	75.0	91.0	200.0	25.0	44.0	100.0
Ni	Starfish	150	270	500	5	150	500	5	13	50
	Snail	300	540	1000	50	72	100	10	16	25
	Sandworm	200	320	500	10	25	200	10	25	50
	Crab	35	130	500	35	47	100	10	30	50
	Clam	500	700	1000	100	320	500	10	112	200
	Fish	400	>400	>400	250	350	400	50	150	250

Table 4. Interpolated LC-25 and LC-75 values at 168 hr for 5 metals and 6 species of estuarine macrofauna at 20°C and 20 o/oo salinity. Values are in ng metal added initially per L of seawater

	Hg		Cd		Zn		Cr		Ni	
	LC-25	LC-75	LC-25	LC-75	LC-25	LC-75	LC-25	LC-75	LC-25	LC-75
Clam	0.002	0.011	0.27	1.90	1.80	3.95	6.30	20.50	35.50	151.00
Starfish	0.014	0.052	0.27	1.90	1.55	3.40	7.20	14.30	8.25	26.00
Crab	0.023	0.081	0.27	1.20	1.45	3.20	1.67	7.60	17.60	38.50
Sandworm	0.039	0.088	0.27	1.90	2.00	3.40	0.27	1.93	16.10	35.50
Snail	0.270	1.900	12.80	20.50	6.10	15.40	7.20	14.30	12.70	20.00
Fish	0.290	0.900	10.80	33.50	23.50	80.00	33.50	67.00	88.00	195.00

concentrations, in mg of metal per L added initially ranged between 0.004 and 0.8 for mercury, 0.7 and 40.0 for cadmium, 0.2 and 52.0 for zinc, 0.7 and 44.0 for hexavalent chromium, and 13.0 and 150.0 for nickel (Table 3). These data demonstrate clearly that, under the experimental conditions, Hg^{2+} was the most toxic metal tested and Ni^{2+} the least toxic; Cd^{2+}, Zn^{2+}, and Cr^{+6} were intermediate in toxicity. Mummichogs were consistently the least sensitive species tested; other species exhibited a wide range of sensitivities to all metals examined.

Despite large numerical differences in LC values listed between metals and between species it would appear, on the basis of interpolated LC-25 (168 hr) and LC-75 (168 hr) values (Table 4), that the toxic action for all metals and species tested was confined to the same relatively narrow range. For example, the mean ratio of LC-75 (168 hr) to LC-25 (168 hr) for mercury was 5.02 (range 2.3 to 7.0); for cadmium this was 6.02 (range 1.6 to 7.0); for chromium 4.20 (range 2.0 to 7.1); for zinc 2.82 (1.6 to 3.4); and for nickel 3.12 (1.6 to 4.3). A similar pattern was demonstrable between species: mean LC-75/LC-25 ratios at 168 hr for the 5 metals were; for clam 4.46; for starfish 3.60; for crab 3.38; for sandworm 4.04; for snail 2.94; and for fish 2.76. As was true for LC-75/LC-25 ratios for individual metals, there was considerable overlap among species in ranges for this parameter.

Discussion

In general, the rank order of toxicity of metals tested was:

$$Hg^{2+} >> Cd^{2+} > Zn^{2+} > Cr^{+6} >> Ni^{2+}$$

Most investigators agree that Hg is the most toxic element in this series and Ni the least toxic. However, there is considerable variation in rank order among aquatic species for Cd^{2+}, Zn^{2+}, and Cr^{+6} (Anderson 1946, Doudoroff and Katz 1953, Cabejszek and Stasiak 1960, Portmann 1968, Bryan 1971, Calabrese *et al.* 1973, Calabrese and Nelson 1974, Bernhard and Zattera 1975, Eisler 1977). These variations appear to us to be related largely to physicochemical properties of the test medium employed by various investigators, with increasing toxicity positively correlated with decreasing salinity and increasing temperature (Eisler 1973, Vernberg *et al.* 1974, Eisler and Wapner 1975, Eisler 1977). It is also probable that variability in resistance under laboratory conditions to heavy metals is a function of biological availability to toxicants as well as inherent genetic differences. For example, unpublished studies that we conducted with adults of quahaug clam, *Mercenaria mercenaria*, under conditions identical to those reported herein yielded LC (168 hr) values for Hg^{2+}, Cd^{2+}, Cr^{+6}, Ni^{2+}, and Zn^{2+} that were several orders of magnitude higher than the most resistant species assayed. Because quahaugs kept their valves tightly shut during the entire exposure period, we attributed their resistance primarily to lack of toxicant exposure.

The LC-50 (168 hr) value of 0.004 mg/L for HG^{2+} and softshell clam in this account suggests that this species is the most mercury-sensitive marine organism tested to date. By comparison the 8-day LC-50 value for larvae of fiddler crab, *Uca pugilator*, under thermal and saline stress is 0.180 mg/L of Hg^{2+} (Vernberg *et*

al. 1974); for oyster embryos, *Crassostrea virginica*, the LC-50 (48 hr) value was 0.0056 mg/L HG^{2+} (Calabrese *et al.* 1973).

Sandworm was the least resistant species tested herein to hexavalent chromium (LC-50, 168 hr = 0.7 mg/L). This is consistent with the finding that polychaete annelids are more sensitive to chromium than other groups of marine macrofauna (Oshida *et al.* 1976). LC-50 values for Cr^{+6} and adults of the polychaete *Neanthes arenaceodentata* ranged from 2.2 to 4.3 mg/L in 96 hrs and 1.4 to 1.9 in 7 days; for *Capitella capitata* exposed for one generation the LC-50 (28 day) value was 0.28 mg/L Cr^{+6} (Oshida *et al.* 1976). *Neanthes* populations subjected continuously for three generations to 0.10 mg/l Cr^{+6} were unable to reproduce, with reduction in brood size observed at concentrations of 0.0125 mg/L Cr^{+6} and higher (Oshida *et al.* 1976).

Cadmium LC values, as was true for those of other metals tested in this study, decreased markedly between 96 and 168 hr. It has been demonstrated that 168 hr is not sufficient to evaluate adequately the toxicological properties of cadmium to selected species of marine biota and that a minimal period of exposure and post-observation is 7 weeks for teleosts (Eisler 1971) and 17 weeks for molluscs (Shuster and Pringle 1968). The relation between LC values derived from acute toxicity studies with cadmium and other metals to those produced from long-term chronic exposures is relatively well-documented for various freshwater species when compared to marine [illegible] (Eisler 1973, Eisler and Wapner 1975). However, additional research effort in this subject area is clearly warranted.

Our results indicate that hermit crab is the most sensitive species tested to zinc with an LC-50 (168 hr) value of 0.2 mg/L. Crustaceans appear to be one of the more sensitive groups to zinc; for example, the LC-50 (150 hr) value for brine shrimp is 0.100 mg Zn^{2+}/L (Brown and Ahsanullah 1971). But other marine groups are also sensitive to zinc at low levels: *Ophryotrocha*, a polychaete annelid, has an LC-50 (25 hr) of 0.100 mg Zn^{2+}/L (Brown and Ahsanullah 1971) and oyster embryos exhibit an LC-50 (48 hr) value of 0.310 mg/L (Calabrese *et al.* 1973). These data reinforce the observation that many species of taxonomically and ecologically divergent groups are required in order to evaluate in a satisfactory manner potentially hazardous compounds.

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